U.S. DEPARTMENT OF COMMERCE
National Technical Information Service

PB-261 499

Preliminary Study Comparative Winter Survival of Fecal Bacteria in Subarctic River

Environmental Protection Agency, College, Alaska

May 74

PB 261 499

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U. S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
4. TITLE AND SUBTITLE Preliminary Study Comparative Winter Survival of Fecal Bacteria in Subarctic River - Working Paper No. 28		5. REPORT DATE May 1974 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Dale J. Van Donsel; Ronald C. Gordon; Charlotte V. Davenport		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Associate Laboratory of National Environmental Research Center Corvallis, Oregon Office of Research and Development		10. PROGRAM ELEMENT NO. 11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS U.S. Environmental Protection Agency Arctic Environmental Research Laboratory College, Alaska		13. TYPE OF REPORT AND PERIOD COVERED 14. SPONSORING AGENCY CODE

15. SUPPLEMENTARY NOTES

16. ABSTRACT

A preliminary investigation of Salmonella survival in a subarctic Alaskan river was conducted during March 1973, when there was total ice cover and 0°C water temperature. Since most of the domestic pollution entered the river from one source and there were no additional sources downstream, it was possible to examine survival without interruption for seven days flow time. Six Salmonella serotypes were isolated, and salmonellae were still recoverable both quantitatively and qualitatively after seven days flow time in the presence of 330 total coliforms/100 ml and 60 fecal coliforms/100 ml.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES 30
	20. SECURITY CLASS *(This page)*	22. PRICE 4.00

EPA Form 2220-1 (9-73)

PRELIMINARY STUDY

COMPARATIVE WINTER SURVIVAL OF FECAL BACTERIA IN A SUBARCTIC RIVER

by

Dale J. Van Donsel

Ronald C. Gordon

Charlotte V. Davenport

WORKING PAPER NO. 28

U. S. Environmental Protection Agency
Arctic Environmental Research Laboratory
College, Alaska

Associate Laboratory of
National Environmental Research Center
Corvallis, Oregon
Office of Research and Development

May 1974

A Working Paper presents results of investigations which are, to some extent, limited or incomplete. Therefore, conclusions or recommendations, expressed or implied, are tentative. Mention of commercial products or services does not constitute endorsement.

ABSTRACT

A preliminary investigation of Salmonella survival in a subarctic Alaskan river was conducted during March 1973, when there was total ice cover and 0°C water temperature. Since most of the domestic pollution entered the river from one source and there were no additional sources downstream, it was possible to examine survival without interruption for seven days flow time. Six Salmonella serotypes were isolated, and salmonellae were still recoverable both quantitatively and qualitatively after seven days flow time in the presence of 330 total coliforms/100 ml and 60 fecal coliforms/100 ml.

TABLE OF CONTENTS

	PAGE
INTRODUCTION	1
MATERIALS AND METHODS	4
RESULTS AND DISCUSSION	12
CONCLUSIONS	19
REFERENCES	20

LIST OF FIGURES

NUMBER		PAGE
1	Map of the lower Tanana River showing the location of sample stations	5
2	Gasoline powered ice auger being used to cut through ice at each sample station	6
3	Samples for quantitative analysis of salmonellae and fecal indicator bacteria being dipped from ice hole	7
4	Setting up tripod to support line with attached qualitative Salmonella sampler and station marker flag	9
5	Method of retrieving line with attached qualitative Salmonella sampler	10

LIST OF TABLES

NUMBER		PAGE
1	Salmonellae/Liter and Fecal Indicator Bacteria/ 100 ml of Sample Obtained at Each Sample Station During the March 21, 1973 Sample Run	13
2	Salmonellae/Liter and Fecal Indicator Bacteria/ 100 ml of Sample Obtained at Each Sample Station During the March 27, 1973 Sample Run	14
3	Salmonella Serotypes Isolated at Each Sample Station from Samples Used for Quantitative and Qualitative Analysis	16
4	Most Frequently Isolated Salmonella Serotypes in the United States During 1972 (4)	17

INTRODUCTION

Decreasing the suspending medium temperature has shown to increase survival time of enteric bacteria and viruses (1, 9, 11, 16, 17, 19, 20). Survival time appears to increase continuously as the temperature decreases, so the longest survival would be expected when the temperature is at or near 0°C. However, most of the survival studies described in these reports were conducted in the laboratory using pure cultures, rather than directly in rivers receiving the enteric microorganisms discharged in effluent from waste treatment systems. Thus, information to fill the practical need for survival time parameters, as stated by Berg et al. (3) in 1966, still remains to be obtained. It has also been stated that coliforms must be quantitatively related to the presence of enteric pathogenic bacteria if coliform enumeration is to continue as a useful criterion of health risk from polluted waters (17).

Increased enteric microogranism survival at low suspending medium temperatures becomes more significant when viewed in relation to current treated wastewater disinfection practice. Recent studies (12, 13) have demonstrated that chlorine disinfection at low effluent temperatures may not effectively remove the fecal indicator bacteria before the effluent is released into the receiving water. Therefore, disinfection under these circumstances may not provide an adequate barrier to the spread of disease caused by enteric pathogenic bacteria. There is also very little chance that enteric viruses are being removed in the disinfection process.

In an effort to obtain low temperature survival time data, a survival study was conducted in a subarctic Alaskan river (11). This study demonstrated that a significant number of total coliforms, fecal coliforms and enterococci remained viable after seven days flow time when the water

temperature was essentially 0°C under total ice cover. The fecal coliform results were compared with temperate climate winter data (2), and the percent survival was found to be approximately five times greater at 0°C than at the warmer temperatures. That study did not include salmonellae, so quantitative survival relationships are not available.

In 1911, Ruediger (20) demonstrated that salmonellae survived in much greater numbers for a much longer period in an ice covered river than during warm weather. He used pure culture suspensions of the bacteria in celloidin sacs which he placed in the river and sampled at various time intervals. More recently, Gallagher et al. (9) found salmonellae to be extremely persistent in the Red River under ice cover. They isolated two Salmonella serotypes 73 miles and four days travel time downstream from the outfall source. In addition, salmonellae were consistently isolated from river water when there were 1000 or more fecal coliforms/100 ml (9), and were occasionally isolated when the fecal coliforms ranged from 4 to 20/100 ml (5, 6, 8, 9, 21).

Approximately 36 percent of all surface waters examined throughout the world have been found to contain enteric viruses (1). Even though enteric viruses have been implicated as the etiological agent in numerous diseases, infectious hepatitis virus is the only one for which definite epidemiological evidence has been obtained showing water as the mode of transmission (17). However, the extended survival of viruses in water makes this a potential route for viral disease transmission (1).

The need for survival time data and quantitative relationships in arctic and subarctic rivers, where the water temperature is 0°C for about six months each year, cannot be over-emphasized since low temperatures enhance enteric microorganism survival causing an increased potential

health hazard downstream. As a result, this preliminary investigation was conducted to determine whether salmonellae could be isolated at the same stations where fecal indicator bacteria had previously been isolated and to establish, if possible, a quantitative relationship under low temperature conditions.

MATERIALS AND METHODS

Selection of River to be Studied

The reach of the Tanana River studied is shown in Figure 1. The description of the river and reasons for selecting this particular river for study have been described previously (11).

Sample Station Selection and Sampling Schedule

Nine sample stations were selected (Figure 1). T-100 through T-800 are essentially in the same locations as previously described (11). Station T-900 was selected to obtain background data upstream from all known sources of domestic pollution.

Two sampling trips were conducted during this preliminary study. All stations were visited on March 21, 1973, for the purpose of collecting samples for quantitative fecal indicator bacteria and Salmonella enumeration, and placing long term qualitative Salmonella samplers (Moore swabs). A second trip was made on March 27, 1973, to obtain another set of samples for quantitative analysis, and to retrieve the swabs.

Sampling Techniques

A gasoline powered ice auger was used to cut through the 1-1/2 to 4 feet of ice encountered at each sample station (Figure 2). Ice chips were skimmed from the water surface, and the samples for quantitative analysis collected by dipping from the water surface (Figure 3). Samples for fecal indicator bacteria enumeration were collected in sterile, wide mouthed, screw capped, 1-liter, polypropylene containers. These containers were transported in ice chests to minimize freezing problems.

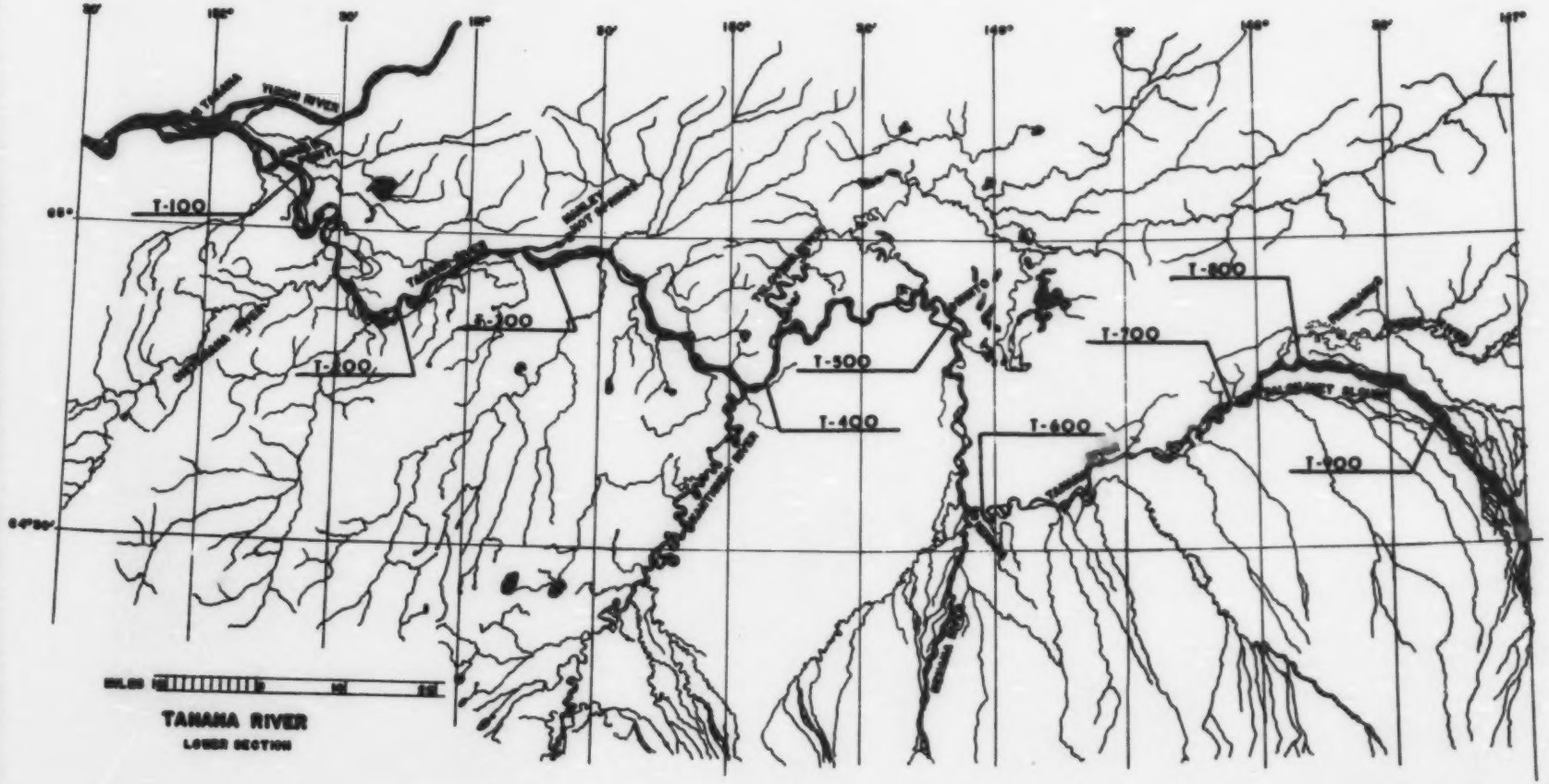

Figure 1. Map of the lower Tanana River showing the location of sample stations

Figure 2. Gasoline powered ice auger being used to cut through ice at each sample station

Figure 3. Samples for quantitative analysis of salmonellae and fecal indicator bacteria being dipped from ice hole

For Salmonella quantitation, samples were collected in 5-gallon Cubitainers. There appeared to be little or no ice formation in these large volume samples. Qualitative Salmonella samples were obtained with the gauze swabs as described by Spion (22). After the samples for quantitative analysis were obtained, the line with the gauze swabs attached was lowered through the hole in the ice, and attached to a tripod for support while the hole was freezing closed (Figure 4). The tripod also supported a flag which marked the sample station. In order to retrieve the gauze swabs after a week in the water, it was necessary to cut another hole in the ice just downstream from the first hole. A steel rod with a hook on the end was used to catch the line and pull it up through the new hole (Figure 5). The guaze swabs were placed in 18-ounce Whirl-Pak bags, and transported to the laboratory in ice chests to minimize freezing.

Fecal Indicator Bacteria Enumeration

Total coliforms, fecal coliforms and fecal streptococci were enumerated by the membrane filter method (13). All sample volumes were filtered in triplicate, and volumes up to 200 ml were used in an effort to obtain numbers of colonies on each filter in the statistically valid range.

Quantitative and Qualitative Salmonella Enumeration

The presence of salmonellae was quantitatively determined by the most probable number (MPN) procedure. The diatomaceous earth method of Hammarstrom and Ljutov (14) was used to concentrate the water samples. Sterile diatomaceous earth (0.5 gm/l) was added to the water. For convenience the membrane filters were removed from sterile, disposable, membrane filter holders (Falcon Plastics, number 7103) and replaced with filter pads of the type accompanying membrane filters. A slurry of 0.5 gm diatomaceous earth was

Figure 4. Setting up tripod to support line with attached qualitative Salmonella sampler and station marker flag

Figure 5. Method of retrieving line with attached qualitative Salmonella sampler

spread on the filter pad, and 12 liters of sample was vacuum filtered. To remove substances that caused erratic salmonellae isolation, the diatomaceous earth plug on the pad was washed five times with sterile buffered distilled water and three times with Dulcitol Selenite Enrichment Medium (DSE) (18). The concentrate plug was then removed from the filter pad and homogenized in a small amount of DSE. Additional DSE was added to bring the total concentrate volume to 120 ml (100:1 concentration of the original sample). The concentrate was then thoroughly mixed, and one 50-ml, five 10-ml, five 1-ml and five 0.1-ml portions were immediately planted into the MPN series in DSE and incubated at 41.5°C. These portions were equivalent to 5,000, 1,000, 100 and 10 ml volumes of the original sample. After 24 and 48 hours of incubation, material from the MPN tubes was streaked on Hektoen Enteric Agar (HE) and Xylose Lysine Desoxycholate Agar (XLD), and incubated for 24 hours at 35°C.

For the qualitative determination of salmonellae, each gauze swab was divided into three equal portions. One portion was placed in a flask of DSE and two in Tetrathionate Broth. Incubation was at 41.5°C and streaks were made on HE and XLD at 24 and 48 hours with incubation at 35°C.

Suspect Salmonella colonies on HE and XLD were picked to Triple Sugar Iron Agar (TSIA) slants. Colonies producing reactions indicative of salmonellae in TSIA tubes were further screened for urease, indole and lysine decarboxylase production before being identified by the serological methods of Edwards and Ewing (7). Additional biochemical tests were performed only if warranted by questionable screening or serological results.

RESULTS AND DISCUSSION

The objectives of this limited preliminary investigation were: [1] to determine whether salmonellae could be isolated as many days flow time downstream from any source of domestic pollution as previously isolated fecal indicator bacteria (11), and; [2] to establish whether or not a quantitative relationship exists between salmonellae and fecal indicator bacteria. Because this quantitative relationship was to be based on the number of bacteria/unit volume of river water, discharge measurement were not necessary at any station.

The results presented in Tables 1 and 2 show that the numbers of fecal indicator bacteria decreased progressively downstream from the T-700 to the T-100 station in a manner comparable with earlier results (11). The T-700 station (Figure 1) is located downstream from the last source of domestic pollution while T-900 is upstream from any source. The fecal coliform numbers were lower than expected from the previous study. However, the m-Fecal Coliform Broth Base (BBL lot #204625) may have been a contributing factor. This medium gave a final reaction pH of 7.2, which is within the accepted 7.2-7.6 pH range. On further checking, it was found that the broth base had a pH of about 6.4 before rosalic acid addition and heating. This is 0.5 to 0.7 pH units lower than obtained with other broth base lots from the same manufacturer. Fecal coliform recovery from unchlorinated primary sewage effluent was examined using the above mentioned broth base lot, and recovery was compared directly with two other lots from the same manufacturer. The results indicated that the broth base used during this study recovered only 48-60 percent as many fecal coliforms as the other two lots. This suggested that the fecal coliform numbers reported in Tables 1 and 2 may be considerably lower than the actual number/unit volume present in the river.

TABLE 1

Salmonellae/Liter and Fecal Indicator Bacteria/100 ml of Sample Obtained at Each Sample Station During the March 21, 1973 Sample Run

Station No.	Total Coliforms (No./100 ml)	Fecal Coliforms (No./100 ml)*	Fecal Streptococci (No./100 ml)	Salmonellae		
				No./liter MPN	95% Confidence Limits No./liter	
T-900	<1§	<1§	<0.5§	0.1#	-----	---
T-800	2300	130†	43	0.1	<0.05	0.4
T-700	9600	1700	130	<0.1#	-----	---
T-600	1700	89†	9†	0.1	<0.05	0.4
T-500	920	160	15†	0.1	<0.05	0.4
T-400	920	110	15†	0.1	<0.05	0.4
T-300	370	64	9†	0.8	0.2	1.9
T-200	410	36	11†	1.7	0.5	4.7
T-100	320	34	13†	<0.1#	-----	---

* These numbers are probably 48-60 percent of the actual number; see text for explanation.

§ No colonies on any filter when triplicate filters were examined.

No salmonellae isolated from any water volume examined.

† Average of less than 20 colonies per filter when triplicate filters were examined.

TABLE 2

Salmonellae/Liter and Fecal Indicator Bacteria/100 ml of Sample Obtained at Each Sample Station During the March 27, 1973 Sample Run

Station No.	Total Coliforms (No./100 ml)	Fecal Coliforms (No./100 ml)*	Fecal Streptococci (No./100 ml)	Salmonellae		
				No./liter MPN	95% Confidence Limits No./liter	
T-900	---**	--**	-**	---**	-----	---
T-800	3600†	480	73	<0.1#	-----	---
T-700	6600	170†	50	0.3	<0.05	0.8
T-600	1600	64†	26	0.1	<0.05	0.4
T-500	2300	260	26	0.1	<0.05	0.4
T-400	960	120	12	0.3	<0.05	0.8
T-300	470	43	10	0.1	<0.05	0.4
T-200	630	63†	21	0.1	<0.05	0.4
T-100	330	60†	6†	0.3	<0.05	0.8

* These numbers are probably 48-60 percent of the actual number; see text for explanation.

** No samples obtained from this station because of open water.

No salmonellae isolated from any water volume examined.

† Average of less than 20 colonies per filter when triplicate filters were examined.

Salmonellae were quantitatively measurable in samples from the T-800 through T-100 stations during one or both sampling trips (Tables 1 and 2). Since these were the same stations at which fecal indicator bacteria were also found, it is apparent that low indicator bacteria numbers do not preclude quantitative measurement of salmonellae. The most probable number (MPN) of salmonellae present at the various sample stations suggests that the numbers of these bacteria do not decrease with flow time. However, the 95 percent confidence limits of the MPN indicate that the salmonellae may be decreasing in a manner similar to the indicator bacteria. A much more detailed study must be conducted before relative survival rates and quantitative relationships between salmonellae and fecal indicator bacteria can be established.

When Salmonella isolates from both quantitative and qualitative samples were examined, six serotypes were found (Table 3). Four serotypes were found in the quantitative samples and five were obtained from the swabs. S. thompson was the only serotype isolated at every station from T-700 to T-100 and was the most frequent isolate from quantitative samples. Thus. S. thompson was probably present in much larger numbers than the other salmonellae. Insufficient data are available to ascertain the numerical relationship of the other isolates, or to determine if survival characteristics vary among the serotypes.

The 10 most frequently isolated Salmonella serotypes from human and nonhuman sources during 1972 are shown in Table 4. Three of the serotypes found in the Tanana River were among those most frequently isolated from human sources and three from nonhuman sources. S. typhimurium and S. derby appear under both headings, while S. thompson was ranked among those most frequently isolated from human sources and S. senftenberg from nonhuman sources.

TABLE 3

Salmonella Serotypes Isolated at Each Sample Station From Samples Used for Quantitative and Qualitative Analysis

Station Number	Quantitative Samples		Qualitative Samples
	March 21, 1973	March 27, 1973	
T-900	None	---------**	---------**
T-800	*derby*	None	*derby*
T-700	None	*thompson*	---------*
T-600	*thompson*	group B non-motile	*thompson* *senftenberg* *typhimurium* *derby*
T-500	*thompson*	*thompson*	----------*
T-400	*thompson*	*thompson*	----------*
T-300	*thompson*	*thompson*	----------*
T-200	*thompson*	*thompson*	*thompson* *senftenberg*
T-100	None	*thompson* *senftenberg*	*thompson* *paratyphi B* *typhimurium*

** No samples obtained from this station because of open water.

* Swab not recovered.

TABLE 4

Most Frequently Isolated Salmonella Serotypes in the United States During 1972 (4)

Rank	Source	
	Human	Nonhuman
1	typhimurium*	typhimurium*
2	newport	oranienburg
3	enteritidis	senftenberg*
4	infantis	saint-paul
5	heidelberg	newport
6	saint-paul	anatum
7	thompson*	montevideo
8	derby*	eimsbuettel
9	oranienburg	derby*
10	javiana	heidelberg

*Isolated from Tanana River.

The results presented in Table 3 indicate that the method used to recover the gauze swabs was not entirely satisfactory since the swabs were not recovered from four stations. The problem was apparently caused by the insulating snow layer having been removed from the ice around the hole through which the line and attached swabs were lowered. During the ensuing week, a cone of ice developed below the lower edge of the ice cover and encased the line on which the swabs were attached. The cone extended deeper than could be reached with the hook so it was impossible to retrieve the swabs. Efforts are currently in progress to develop a means of overcoming this serious problem.

Circumstances did not permit the examination of enteric viruses during this preliminary investigation. In view of the available information about extended virus survival at low water temperatures (1), isolation and identification of enteric viruses should constitute a major effort during any detailed enteric microorganism survival study. Field methods for concentration of viruses from large volumes of water are available (15), and one or more of these should be applicable under low temperature conditions, making a virus survival study feasible.

CONCLUSIONS

1. Salmonella serotypes survive for at least seven days flow time downstream from the last source of domestic pollution when the water temperature is 0°C and there is total ice cover.

2. After seven days flow time, a variety of Salmonella serotypes are still recoverable both quantitatively and qualitatively when total and fecal coliforms are present in low numbers.

3. The results from this preliminary study were too few to determine the extent to which fecal indicator bacteria numbers would indicate Salmonella density. The results do suggest that a survival relationship may exist. However, a more detailed study is necessary to assess the quantitative relationship.

4. Enteric virus survival should also be examined under the same low temperature conditions.

REFERENCES

1. Akin, W. E., W. H. Benton, and W. F. Hill. 1971. Enteric Viruses in Ground and Surface Waters: A Review of Their Occurrence and Survival. p. 59-70. In V. Snoeyink and V. Griffin (ed.), Proceedings of the 13th Water Quality Conference, Virus and Water Quality: Occurrence and Control.

2. Ballentine, R. K. and F. W. Kittrell. 1968. Observation of Fecal Coliforms in Several Recent Stream Pollution Studies. In Proceedings of the Symposium on Fecal Coliform Bacteria in Water and Wastewater. Burear of Sanitary Engineering, California State Department of Public Health.

3. Berg, G., P. V. Scarpino, and D. Berman. 1966. Survival of Bacteria and Viruses in Natural Waters. Federal Water Pollution Control Administration, Cincinnati.

4. Center for Disease Control. 1973. Salmonella Surveillance Annual Summary. Department of Health, Education and Welfare, Atlanta.

5. Dutka, B. J. 1973. Coliforms Are an Inadequate Index of Water Quality. J. Environmental Health. 36:39-46.

6. Dutka, B. J. and J. B. Bell. 1973. Isolation of Salmonellae from Moderately Polluted Waters. J. Water Pollution Control Federation. 45:316-324.

7. Edwards, P. R. and W. H. Ewing. 1972. Identification of Enterobacteriaceae, 3rd Ed. Burgess Publishing Company, Minneapolis.

8. Fair, J. F. and S. M. Morrison. 1967. Recovery of Bacterial Pathogens from High Quality Surface Water. J. Water Resources Research. 3:799-803.

9. Gallagher, T. P., J. E. Hagan, N. A. Thomas, and D. F. Spino. 1965. Report on Pollution of Interstate Waters of the Red River of the North (Minnesota-North Dakota). Department of Health, Education and Welfare, Cincinnati.

10. Geldreich, E. E. and N. A. Clarke. 1971. The Coliform Test: A Criterion for the Viral Safety of Water. p. 103-109. In V. Snoeyink and V. Griffin (ed.), Proceedings of the 13th Water Quality Conference, Virus and Water Quality: Occurrence and Control.

11. Gordon, R. C. 1972. Winter Survival of Fecal Indicator Bacteria in a Subarctic Alaskan River, Pub. No. EPA-R2-72-01. Environmental Protection Agency, College, Alaska.

12. Gordon, R. C. and C. V. Davenport. 1973. Batch Disinfection of Treated Wastewater With Chlorine at Less Than 1°C, Pub. No. EPA-660/2-73-005. Environmental Protection Agency, College, Alaska

13. Gordon, R. C., C. V. Davenport, and B. H. Reid. 1973. Chlorine Disinfection of Treated Wastewater in a Baffled Contact Chamber at <1°C, Working Paper No. 21. Environmental Protection Agency, College, Alaska.

14. Hammarstrom, E. and V. Ljutov. 1954. Concentration Technique for Demonstrating Small Amounts of Bacteria in Tap Water. J. Acta Pathologica et Microbiologica Scandinavica. 35:365-369.

15. Hill, W. F., E. W. Akin, and W. F. Hill, Jr. 1971. Detection of Viruses in Water: A Review of Methods and Application. p. 17-42. In V. Snoeyink and V. Griffin (ed.), Proceedings of the 13th Water Quality Conference, Virus and Water Quality: Occurrence and Control.

16. McFeters, G. A. and D. G. Stuart. 1972. Survival of Coliform Bacteria in Natural Waters: Field and Laboratory Studies with Membrane-Filter Chambers. J. Applied Microbiology. 24:805-811.

17. Mechalas, B. J., K. K. Hekimian, L. A. Schinazi, and R. H. Dudley. 1972. Water Quality Criteria Data Book, Vol. 4, An Investigation into Recreational Water Quality, Pub. No. 18040 DAZ 04/72. Environmental Protection Agency, Washington, D.C.

18. Raj, J. 1966. Enrichment Medium for Selection of Salmonella from Fish Homogenate. J. Applied Microbiology. 14:12-20.

19. Rudolfs, W., L. L. Falk, and R. A. Ragotzkie. 1950. Literature Review on the Occurrence and Survival of Enteric, Pathogenic, and Relative Organisms in Soil, Water, Sewage, and Sludges, and on Vegetation. J. Sewage and Industrial Wastes. 22:1261-1281.

20. Ruediger, G. F. 1911. Studies on the Self-Purification of Streams. J. American Public Health Association. 1:411-417.

21. Smith, R. J., R. M. Twedt, and L. K. Flanigan. 1973. Relationships of Indicator and Pathogenic Bacteria in Stream Waters. J. Water Pollution Control Federation. 45:1736-1745.

22. Spino, D. F. 1966. Elevated-Temperature Technique for the Isolation of Salmonella From Streams. J. Applied Microbiology. 14:591-596.

23. Van Donsel, D. J. and E. E. Geldreich. 1971. Relationships of Salmonellae to Fecal Coliforms in Bottom Sediments. J. Water Research. 5:1079-1087.

S. GOVERNMENT PRINTING OFFICE: 1974—697-595/54 REGION 10